## Remarks

### I. Objections to the Specification and Claims

The examiner objects to the text of the application for omission of sequence identifiers in the text, for inclusion of hyperlinks, and for improperly identified trademarks. The examiner further indicates that the brief description of the drawings should be amended to include reference to Figures 6A and B and Figures 19A, B and C. The specification has been amended as set out in the substitute specification to correct these clerical errors in the text.

The examiner objects to claims 57-58 and 59-60 for having duplicate subject matter, objects to claim 50 for a typographical error and objects to claims 53 and 54 for improper sequence compliance. The claims have been amended accordingly thereby obviating the objections.

The amendment to the specification and claims includes no new matter.

Applicants note the examiner's objection to Applicants' priority claim. As evidenced by the discussion of the cited documents below, the priority claim is not necessary to overcome the disclosure of the documents so further discussion of the merits of the priority claims is not required. Applicants will address the issue in other applications if it is ever necessary.

### II. IDS and Biological Deposit

Applicants acknowledges the examiner's comments regarding the IDS. Applicants submit herewith missing reference BBBB on the 1449 form of July 2, 2004.

Applicants also acknowledge the examiner's comments regarding the omitted sequence identifiers in the specification and the possibility of depositing the hybridomas referred to in the claims. Applicants submit that amendment to the specification addresses the examiner's concerns.

## III. Examiner's Grounds for Rejection

In the Office Action, the examiner variously rejected pending claims 1-79 and 81-109 under 35 USC §112, second paragraph, for asserted indefiniteness, and under 35 USC §112, first paragraph, for assertedly lacking enablement and written description in the specification. The claims were also variously rejected under 35 U.S.C. § 103(a) as follows: claims 1-12, 14-23, 25-28, 31-47, 52-55, 57, 58, 61-63, 65, 68, 69, 71, 72, 76-79, and 109 as obvious over Shan et al., *J Immunol* 162:6589-95, 1999 (hereinafter "Shan") in view of Pluckthun, US Patent 6,815,540 (herinafter "Pluckthun"); claims 1, 56, 65, 70-72 under 103(a) as obvious over Shan and Pluckthun in view of Bodmer, US Patent 5,677,425 (hereinafter "Bodmer"); claims 1, 63, 66, and 82 as obvious over of Shan and Pluckthun further in view of Bodmer and Morrison, US Patent 6,284,536 (hereinafter "Morrison"); and claims 1, 64, 67, 73-75, 77 and 81 as obvious over Shan and Pluckthun further in view of Roux et al., *J Immunol* 161:4083-90, 1998 (hereinafter "Roux").

Applicants respectfully request reconsideration in light of the amendments and response filed herein.

## IV. The Rejection of Claims 3, 13-16, 29 and 30 under 35 U.S.C. §112, Second Paragraph, Should Be Withdrawn

The examiner rejects claim 3 as assertedly indefinite in the recitation of the phrase "the one or more amino acid substitution or deletion in said heavy chain variable

region" as lacking antecedent basis. Claim 3 has been amended to correct this clerical omission thereby obviating the rejection.

With respect to the term "des-leucine" in claim 13, the designation "des" preceding the name of the amino acid indicates that that particular amino acid has been deleted and no amino acid has been substituted in its place (See, e.g., p 555, col. 1, *J Biol Chem* 242:555-557, 1967, submitted herewith). This designation is used frequently in the art.

The examiner objects to claims 14-16 as assertedly indefinite for reciting the phrase "an increased recombinant expression or stability" contending that it is not clear how expression or stability can be recombinant. The phrase an "increased recombinant expression" refers to the recombinantly expressed protein having increased expression and stability when expressed recombinantly compared to other proteins when expressed in the same manner. The phrase "recombinant expression" is commonly used in the art, e.g. "recombinant expression vector" (paragraph 441 of the published specification) and would be understood by one of ordinary skill in the art. As such, the claims are clear to a person of ordinary skill.

The examiner objects to claims 29 and 30 as assertedly indefinite inreciting the phrase wherein the single chain Fv is a "hd37 single chain fv, 2h7 single chain fv ", etc. The examiner asserts it is unclear if the single chain protein or scFv is produced by the hybridoma or whether the VH, VL, hinge and other components of the antibody are cloned from a hybridoma. Claims 29 and 30 ultimately depend from claims 1 and 2, respectively, which are directed to a single chain protein having a binding domain such as a scFv. Generally, a single chain Fv comprises the VH and VL domain sequences that are based on the sequences of a particular hybridoma and a linker region. Therefore, claims 29 and 30 refer to the binding domain of a single chain Fv wherein the scFv expresses the VH and VL

regions that are based on the sequences of any one of the hybridoma designations recited in front of the scFv label, such as 2H7 scFv or HD37 scFv. Thus, for example, claim 29 recites a single chain protein having a binding domain which is an scFv, wherein the scFv sequence of the binding domain is the variable chain sequence of the antibody produced by the 2H7 hybridoma. A person of ordinary skill reading the claims would understand this terminology.

## V. The Rejection of Claims 29, 30 and 83-108 under 35 U.S.C. §112, First Paragraph-Enablement, Should Be Withdrawn

The examiner rejects claims 29-30 and 83-108 under 35 USC §112, first paragraph, as allegedly lacking enablement in the specification The examiner asserts that the specification does not teach that the hybridomas set out in the claims are readily available to the public and are reproducible from the written description.

Applicants submit that the specification teaches the sequences of the variable regions of the antibodies produced by the hybridomas described in the specification and that the substitute specification filed herewith sets out the sequences in the sequence listing corresponding to the particular hybridoma variable heavy and light chain regions. For example the sequence of the hybridomas are set out as follows: 2H7 (SEQ ID NO: 21-28); HD37 (SEQ ID NOs: 387-392); G28 (SEQ ID NOs: 309-314); FC2-2 (SEQ ID NOs: 333-338); UCHL-1 (SEQ ID NOs: 347-352); 5B9 (SEQ ID NOs: 116-123); L6 (SEQ ID NOs: 403-408); 10A8 (SEQ ID NOs: 43-48); 2E12 (SEQ ID NOs: 37-42); G19-4 (SEQ ID NOs: 443-444); 1D8 (SEQ ID NOs: 100-105); and 4.4.220 (SEQ ID NOs: 32-36). Therefore, the specification has described the hybridomas sufficiently and enabled one of ordinary skill in the art to make and use the hybridoma sequences to formulate an scFv having the variable region sequences of these hybridomas and also make modifications, such as amino acid substitutions, deletions, etc., to the sequences based on the teachings in the specification.

As such, the rejection of claims 29, 30 and 83-108 under 35 USC §112, first paragraph, enablement, should be withdrawn.

## VI. The Rejection of Claims 1-79 and 81-109 under 35 U.S.C. §112, First Paragraph-Enablement, Should Be Withdrawn

The examiner rejects claims 1-79 and 81-109 under 35 USC §112, first paragraph, as assertedly lacking enablement for a binding-domain Ig fusion protein comprising a binding domain polypeptide which comprises only a light chain variable domain or only a heavy chain variable domain, for a single chain antibody or scFv comprising any amino acid substitution or deletion in position 9, 10, 11, 12, 108, 110, 112 in the VH region or amino acids 12, 80, 81, 83, 105, 106, and 107 in the VL region, or for a modified VH region of a hybridoma sequence set out in the claims. The examiner's various rejections are addressed in the sections below.

### A. The specification does enable single chain proteins comprising only a heavy chain domain or light chain domain

The examiner asserts that the specification does not enable a fusion protein of the invention having only a heavy chain domain or only a light chain domain, and only enables a protein having a heavy chain and a light chain variable regions. The examiner further asserts that a functional antibody binding domain requires both a heavy chain and light chain variable region to get binding to an antigen.

Claims 1 and 77 are directed to single chain proteins having a binding domain comprising a heavy chain variable region, which thereby encompasses a protein having at least a heavy chain variable region and a protein having both a heavy chain variable region and a light chain region. The claims do not recite a protein having only a light chain variable region.

Further, the specification teaches, and it was known in the art at the time of filing, that a certain subset of antibodies are functional having only a heavy chain variable region. For example, paragraph s 45-46 of the published specification, teach that camelid species, including camels, llamas, and alpacas, and certain species of sharks produce antibodies having two heavy chain variable regions and no light chain variable region. See also Nuttall et al., *Curr Pharm Biotechnol.* 1:253-63, 2000 (submitted herewith), and Muyldermans, S. *J Biotechnol.* 74:277-302, 2001 (abstract submitted herewith). Thus, heavy chain-only antibodies are naturally-occurring and may be reproduced by recombinant means as taught in the specification. Further, domain antibodies, a protein construct comprising only a single domain of either a heavy chain or a light chain variable region that retain antigen binding affinity have been disclosed in the art (Ward et al., Nature 341:544-6, 1989) (abstract submitted herewith). As such, it is possible to formulate a single chain protein of the invention having a binding domain comprising at least only a heavy chain variable region without undue experimentation, and provide specific antigen binding, contrary to the examiner's assertion.

Moreover, experimentation is not necessarily undue if it is routine in the art (*In re Wands*, 858 F.2d 731 (Fed. Cir. 1988)). The specification teaches methods to generate antibodies having a variable region of any origin, and the level of skill in the art of recombinant protein engineering is high, such that one of ordinary skill can make a single chain construct of the invention using routine techniques in the art. Further, the specification teaches methods of assaying the binding affinity of the constructs without undue experimentation (see, for example, Example 2). Thus, the experimentation required to make and use a single chain protein of the invention is routine to a worker having ordinary skill in the art, and the rejection of the claims should be withdrawn.

B. The specification does enable a single chain protein of the invention comprising an amino acid substitution in either or both the heavy chain and light chain variable domains

The examiner asserts that the specification is not enabling for a single chain protein having an amino acid substitution in a heavy chain variable region AND a light chain variable region. The examiner asserts that the application does enable substitutions at variable region amino acids 9, 10, 11, 123, 108, 110 and 112 for all amino acids listed in the specification except for des-leucine, and does not further indicate why the specification is not enabled for the substitutions in the light chain variable region. The examiner further asserts that Applicant has not taught which modifications could be predictably made and which choice is likely to be successful.

A stated above in Section IV, "des" and des-leucine are a term of art in the chemical field indicating that the residue at the amino acid position referred to by the designation is missing and no other amino acid has been substitution for it. Applicants have described a modification of the leucine at position 11 wherein the leucine is deleted (paragraph 121). Therefore , the specification does teach substitution with des-leucine.

With respect to the guidance in the specification relating to substitutions in the variable region, Paragraph 346 of the publication describes several publications which describe methods of engineering framework regions and CDR in the antibody variable region, and methods to achieve functional antibodies after modification of these regions, teaching that areas that are in contact with other domains of the antibody (e.g. CH1 or VL if a heavy chain variable region) may be altered in single chain proteins of the present invention not having these binding needs. Additionally, WO92/01787, and WO98/02462 describe residues of the variable regions in which amino acid substitutions may be made. Further, the specification teaches methods for determining whether the constructs having mutations in any

one of these amino acid residues are functional, such as assessing binding to cells expressing the antigen of interest (see Example 2). These methods may be used for any binding molecule of interest.

An Applicant is not required to put into the application that which is known or readily available in the art, for example "the patent need not teach what is well known in the art," (Hybridtech, Inc. v Monoclonal Antibodies, Inc., 802 F.2d 1367 (Fed. Cir. 1986)). The locations of the CDR and framework regions are well-known in the art, and it was known at the time of filing that modification in framework areas and some regions of the CDR were more amenable to modification than other residues. Given the high level of skill in the art of recombinant protein engineering, and the teaching in the specification of how to modify antibody variable regions and methods of screening for binding affinity, one of ordinary skill would be able to make and use a construct of the invention comprising a mutation in either a heavy chain or a light chain variable region in the binding domain of the construct without undue experimentation. Therefore, the rejection of the claims as not enabled should be withdrawn.

C. The specification does enable a single chain protein expressing a binding domain based on a hybridoma scFv further having a modification in the scFv variable region

The examiner asserts that claims 29, 30 and 83-108 can be interpreted to read on scFvs having modifications in the variable region which are produced by hybridomas recited in the claims and in the specification. The examiner alleges that Applicants have not shown that any of the named hybridomas express an antibody comprising a variable domain having the desired modifications.

Applicants submit that the claims are directed to proteins having binding domains comprising variable region sequences that are based on the sequences of the indicated hybridomas, and not to a hybridoma producing the an antibody having the altered variable region. Claims 29 and 30 ultimately depend from claims 1 and 2 which recite a single chain protein having a binding domain polypeptide wherein said binding domain polypeptide is a single chain. Claims 83-108 ultimately depend from claim 77 which recites a similar binding domain as claim 1. Therefore, claims 29 30 and 83-108, when read in light of claim 1 and 2 or 77, recite a single chain protein comprising a binding domain having a sequence based on the sequences of the particular VH and VL regions expressed by the hybridoma.

Further, the specification discloses the sequence of the scFV and the sequences of the variable regions of the hybridomas recited in the claims and describes methods of modifying these sequences using recombinant methods. As such, the specification teaches one of ordinary skill in the art how to make and use the invention claimed in claims 29, 30 and 83-108.

For the reasons stated above, the rejection of claims 1-79 and 81-109 under 35 USC §112, first paragraph, as assertedly lacking enablement, should be withdrawn.

**VII. The Rejection of Claims 1-12, 14-23, 25-28, 31-47, 52-55, 57, 58, 61-63, 66, 65, 68, 69, 71, 72, 76-79 and 109 under 35 U.S.C. §103(a) Should Be Withdrawn**

The examiner rejects claims 1-12, 14-23, 25-28, 31-47, 52-55, 57, 58, 61-63, 66, 65, 68, 69, 71, 72, 76-79 and 109 under 35 USC §103(a) as allegedly obvious over Shan and Pluckthun. The examiner contends that Shan teaches that the constructs it discloses "are amendable to further structural modification" (see page 25 of the Action) and asserts that it

would be obvious for one of ordinary skill in the art to take a protein construct of Shan and modify the protein according to the teachings of Pluckthun.

The rejected claims are dependent from either claims 1 or 77 which are directed to a single chain protein having a binding domain comprising a heavy chain variable region with a modification at position 11 in the heavy chain variable region.

Shan discloses an scFv specific for the CD20 molecule further comprising an hinge, CH2 and CH3 region of the IgG1 antibody. Shan teaches that the length of the linker region in the scFv construct plays a role in the affinity and aggregation of the protein. Shan teaches that additional "structural modifications are underway to generate dimeric scFv which may prove superior to the present constructs" (Page 6594, col. 2). Shan neither discloses nor suggests structural modifications in terms of modification of the variable region to obtain increased expression.

Pluckthun teaches that residues in the variable region of an antibody involved in contact with either its corresponding constant region or with the complementary variable region, may be altered to potentially provide stability and increased recombinant expression of the engineered antibody. Pluckthun describes 16 possible residues in each of the heavy chain and light chain variable region that may be mutated (col. 5, line 66, to col. 6. line 4) and describes the generation of several scFv having one or more mutations in the residues set out in the description. Pluckthun teaches changing residues 84, 87 and/or 89 imparts improved properties (col. 11, lines 20-59).

A worker of ordinary skill in the art reading Shan in view of Pluckthun would not be motivated to generate a construct having a mutation at position 11 in the heavy chain variable region. Pluckthun discloses sixteen different possible modifications in each of the

heavy and light chain variable regions. Of those sixteen, changing residues other than the one at position 11, or changing position 11 in combination with other amino acid substitutions, confers an advantage,. For example, Pluckthun teaches that mutation of residue 84 in the heavy chain confers the greatest improvement in protein expression. One of ordinary skill in the art reading Shan would not be motivated to generate a modified construct having a VH modification at position 11 when Pluckthun teaches toward other modifications. Moreover, the present specification demonstrates that changing the leucine at position 11 of the single chain protein of the invention improves the expression levels of the modified protein (paragraph 632, Example 34), which is unexpected given the results taught in Pluckthun.

A person of ordinary skill in the art would not be motivated by Pluckthun to modify residue 11 in the VH region in a construct set out in Shan to arrive at a single chain protein with improved production. As such, the rejection of claims 1-12, 14-23, 25-28, 31-47, 52-55, 57, 58, 61-63, 66, 65, 68, 69, 71, 72, 76-79 and 109 as obvious over Shan in view of Pluckthun should be withdrawn.

## VIII. The Rejection of Claims 1, 56, 65 and 70-72 under 35 U.S.C. §103(a) Should Be Withdrawn

The examiner rejects claims 1, 56, 65, and 70-72 under 35 USC §103(a) as allegedly obvious over Shan and Pluckthun further in view of Bodmer.

The claims are directed to a single chain protein having a binding domain comprising a variable region with a modification at residue 11, further wherein the Ig region is humanized (claim 56), wherein the connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue (claim 65) or wherein

the connecting region has one cysteine residue, comprises no more than one cysteine residue or wherein the connecting region is altered so that said protein has a reduced ability to dimerize (claims 70-72).

Shan and Pluckthun have been described above. Bodmer describes a tetrameric antibody construct comprising variable regions, a hinge, CH1, CH2 and CH3 regions, wherein the altered antibodies have a reduced number of cysteine residues in the hinge region. Bodmer further discloses that the variable regions of the antibody may be humanized. Bodmer neither discloses nor suggests a construct having the structure of the claimed constructs, nor modification of position 11 of the heavy chain variable region.

As stated above, a worker of skill in the art reading Shan in view of Pluckthun would not be motivated to generate a construct of the invention having a modification at residue 11 in the VH region. A person of ordinary skill in the art reading Bodmer in view of Shan and Pluckthun would not have been led to the single chain proteins recited in claim 1 because none of the three references, alone or in combination, provide motivation to produce a single chain protein having the structure of the claimed proteins possessing a modification at position 11 of the VH as claimed.

Thus, the Examiner has failed to establish a *prima facie* case of obviousness for any of the rejected claims and the rejection of claims 1, 56, 65 and 70-72 under 35 U.S.C. §103(a) as obvious over Shan and Pluckthun in view of Bodmer should be withdrawn.

## IX. The Rejection of Claims 1, 63, 66 and 82 under 35 U.S.C. §103(a) Should Be Withdrawn

The examiner rejects claims 1, 63, 66, and 82 under 35 USC §103(a) as allegedly obvious over Shan and Pluckthun further in view of Bodmer and Morrision.

The claims are directed to single chain proteins of the invention comprising a VH domain having a modification at position 11, further comprising an IgA hinge region, which may modified to contain a particular number of cysteine residues.

Shan, Pluckthun and Bodmer have been described above. Morrison describes modified antibodies having various domains of an IgA antibody, including the hinge, CH1, CH2 or CH3 region of IgA. Morrison neither discloses nor suggests modification of the variable regions to improve protein production or yield or for any other reason.

As stated above, a worker of skill in the art reading Shan in view of Pluckthun would not be motivated to generate a construct of the invention having a modification at residue 11 in the VH region. A person of ordinary skill in the art reading Morrison in view of Shan, Pluckthun and Bodmer would not have been led to the single chain proteins recited in claim 1 because none of the references, alone or in combination, provide motivation to produce a single chain protein having the structure of the claimed proteins possessing a modification at position 11 of the VH as claimed.

Thus, the Examiner has failed to establish a *prima facie* case of obviousness for any of the rejected claims and the rejection of claims 1, 56, 65 and 70-72 under 35 U.S.C. §103(a) as obvious over Shan and Pluckthun in view of Bodmer further in view of Morrison should be withdrawn.

## X. The Rejection of Claims 1, 64, 67, 73-75, 77 and 81 under 35 U.S.C. §103(a) Should Be Withdrawn

The examiner rejects claims 1, 63, 66, and 82 under 35 USC §103(a) as allegedly obvious over Shan and Pluckthun further in view of Roux.

The claims are directed to single chain proteins having a binding domain comprising a heavy chain variable region with a modification at residue 11, further comprising a hinge region derived from either IgE or IgG1, and wherein the connecting region comprises three cysteine residues and one proline residue.

Shan and Pluckthun have been described above. Roux describes antibodies having IgE and IgG1 hinge regions and describes generating hinge regions having proline substitutions at the cysteine residues. Roux neither discloses nor suggests modification of the variable regions nor discloses a single chain protein having the structure of the claimed proteins.

As stated above, a worker of skill in the art reading Shan in view of Pluckthun would not be motivated to generate a construct of the invention having a modification at residue 11 in the VH region. A person of ordinary skill in the art reading Roux in view of Shan and Pluckthun would not have been led to the single chain proteins recited in claim 1 because none of the three references, alone or in combination, provide motivation to produce a single chain protein having the structure of the claimed proteins possessing a modification at position 11 of the VH as claimed.

Thus, the Examiner has failed to establish a *prima facie* case of obviousness for any of the rejected claims and the rejection of claims 1, 56, 65 and 70-72 under 35 U.S.C. §103(a) as obvious over Shan and Pluckthun further in view of Roux should be withdrawn.

**XI. Conclusion**

Applicants submit that the application is in condition for allowance and request notification of the same.

Dated: June 8, 2007

Respectfully submitted,

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